FREE RADICAL SCAVENGING ACTIVITY OF LEAVES OF *BISCHOFIA JAVANICA* BLUME AND *FRAXINUS FLORIBUNDA* WALLICH

Sutharson Lingadurai[1*], Prasanna Kumar Kar[1], Lila Kant Nath[2] Shila.E.Besra[3]and Rajan Veda Siromoni Joseph[3]

[1]Department of Pharmacology, Himalayan Pharmacy Institute, Majhitar, Sikkim-737136, INDIA
[2] Department of Pharmaceutical Sciences, Dibrugarh University, Assam -786004, INDIA
[3] Drug Development Divisions, Indian Institute of Chemical Biology, Kolkata - 700032, INDIA

Summary

Bischofia javanica Blume and *Fraxinus floribunda* Wallich are ethnomedicinally used for many diseases like tuberculosis, ulcers, fracture, dislocation and other inflammatory conditions. Free radicals are the major factors responsible for inflammatory diseases. Therefore in this study free radical scavenging properties of both the plants were studied. DPPH radical, Lipid peroxidation and Hydroxyl radical scavenging properties at the concentration range of 20-320µg/ml of methanolic extract and the isolated compounds Friedeline 3α-acetate (FA) and β-amyrin from *Bischofia javanica* and *Fraxinus floribunda* were investigated. Results revealed that significant free radical scavenging properties of the test drugs. The least IC_{50} values (168.47, 137.90 and 129.85 µg/ml) of β-amyrin in all the assay methods showed the potent scavenging property compared to other test drugs. This study supported the fact of anti-inflammatory drugs may have free radical scavenging properties vice versa.

Key words: *Bischofia javanica, Fraxinus floribunda,* DPPH, Lipid peroxidation and Hydroxyl radical scavenging.

*Corresponding Author:
Dr. Sutharson Lingadurai,
Professor & Head, Department of Pharmacology,
Himalayan Pharmacy Institute, Majhitar, E. Sikkim-737136, INDIA
Tel: +91 9932232464; Fax: +91 3592 246247,
E-Mail: apjak_son@yahoo.co.in, drlsivakani@gmail.com

Introduction

Oxygen free radical induced cellular damage has been implicated in many pathological conditions, like malignancy, ageing process, inflammation and degenerating diseases (1-2). Reactive oxygen species (ROS) have been known to cause tissue injury through covalent bonding and lipid peroxidation (3). Anti-inflammatory drugs could conceivably affect oxidant damage at the sites of inflammation in several ways. They might directly scavenge such reactive oxidants as hydroxyl radical ($OH^{\cdot}$) and hypochlorous acid (OHCl) (4). Anti-oxidants act as major defense against radical-mediated toxicity by protecting the damages caused by free radicals. Plant-derived antioxidants may function as reducing agents and scavengers of free radicals (5). Thus, phytochemicals may combat oxidative stress in human body by maintaining a balance between oxidants and antioxidants.

Bischofia javanica Blume (Euphorbiaceae) commonly known as Bishop Wood. In India it is distributed over the Sub-Himalayan region, Orissa and south-West Coast from Konkan to Nilgiris (6). Traditionally the bark is used for the treatment of Tuberculosis, stomach ulcer, body ache mouth ulcer and for inflammatory conditions (7-9). *Fraxinus floribunda* Wallich (Oleaceae), it is distributed over eastern Himalayas and Khasi hills. Manna is obtained by incision from the stem of the tree and it is used as laxative (10). The barks and leaves of the plant have been traditionally used for the treatment of fracture and dislocation (11). The leaves are employed as diuretic and for the treatment of gout (12). The major phytoconstituents which have been isolated from *Bischofia javanica* were tannin, β amyrins, betulinic acid, friedelan-3α-ol, epifriedelinol, friedelin, luteolin and glucoside, quercetin, beta-sitosterol, stigmosterol, ursolicacid (13-15). Some coumarins have been isolated from the leaves of *Fraxinus floribunda* (16).Both the plants were shown to have potent anti-inflammatory activities in the previous research work (17-18). Therefore in this research work the free radical scavenging activity of these plants were studied to verify the hypothesis of anti-inflammatory drugs may have free radical scavenging property vice versa.

Methods

Plant Material

The leaves of *Bischofia javanica* and *Fraxinus floribunda* were collected from East Sikkim (India). The plant materials were identified and authenticated at Botanical Survey of India (BSI), Sikkim branch and the herbaria were preserved in the institutional museum: HPI /LS/ No.12 and 13

Extraction Procedure

The powdered leaves (5 Kg) were extracted with methanol in soxhlet apparatus at 50-60°C. After exhaustive extraction the extract was concentrated by distilling the solvent for further use. The concentrated extract was kept in the desiccators. Yields of the prepared extracts were 5 %w/w and 3%w/w of the leaf powder of *B.javanica* and *F.floribunda.* The extract was used for the study of preliminary chemical analysis.

Phytochemical Studies

The preliminary phytochemical test of the leaf extracts for the presence of alkaloids, flavanoids, terpenoids, glycosides, saponins and tannins was performed by the standard methods (19-21).

The methanol extract was concentrated, suspended in distilled water and partitioned with petroleum ether, chloroform (22-23). The chloroform soluble fraction was subjected to thin layer chromatographic analysis. The aqueous and petroleum ether fraction did not show any positive pharmacological activities under purview of this investigation and was discarded. he dried solid chloroform fraction of the extract was undertaken for column chromatography using silica gel as the stationary phase. Gradient elution was carried out using n-hexane and ethyl acetate as solvent by increasing the polarity of n-hexane by adding increments of ethyl acetate. (24-25). The isolated compounds were further examined by IR, 1-H, 13-C NMR and Mass spectroscopic techniques for its structure elucidation (26- 27).

Animals

Wistar female albino rats (150-250g) were used in this study**.** They were housed in large propylene cages and kept at 22±2°C in 12 h dark-light cycle. The animals were fed with pellet food and water *ad libitum*. All animals were acclimatized to the laboratory environment for at least one week before the experimental session. The animal experiment was approved by Institutional Animal Ethical Committee (IAEC) No: HPI/07/60/IAEC/0006.

Free Radical Scavenging Activity

DPPH Radical Scavenging Assay

To different concentrations (20, 40, 80,160 and 320µg/ml) of methanol extract of the leaves of *Bischofia javanica* (BJ*), Fraxinus floribunda* (FF) and the isolated constituents Friedeline 3α-acetate (FA) and β-amyrin,1ml of a methanolic solution of 0.2Mm DPPH was added (28). After mixing thoroughly, the mixture was allowed to stand in dark for 30min and the absorbance at 523 nm was measured using methanol for the baseline correction. α-tocopherol was used as a positive control. Readings were taken in quadruplicate. The scavenging activity was expressed as IC_{50} value, which is defined as the concentration (µg/ml) of extract required for inhibiting the formation of DPPH radicals by 50%. The percentage of DPPH radical scavenging activity was calculated.

Lipid Peroxidation Assay

The brains of normal female albino rats of the Wistar strain (age 3months) were dissected and homogenized in chilled 20mM Tris-HCl, pH 7.4. The homogenate was centrifuged at 20,000×g for 15 min at 4°C. A 0.5-ml aliquot of the supernatant was added and incubated with 0.3 ml BJ, FF, FA and β-amyrin at the concentrations of 20, 40, 80,160 and 320µg/ml, 0.1 ml of 10 mM FeSO4 and 0.1 ml of 0.1mM ascorbic acid at 37°C for 1h.

The reaction was then stopped by addition of 0.75 ml of solution of trichloroacetic acid (TCA) and 0.5 ml of 1% (w/v) solution of thiobarbituric acid (TBA). The mixture was heated at 100°C for 45 min. After centrifugation, the precipitated proteins were removed and the color of the malondialdehyde (MDA)-TBA complex in the supernatant was measured at 532 nm (29).

Hydroxyl Radical Scavenging Assay

The hydroxyl radical scavenging activity of for the test drugs were determined by adopting the method of Aruoma et al (30). The assay mixture, in a total volume of 1.2 ml, contained deoxyribose (2.8mM), $FeCl_3$ (25mM), EDTA (100mM), H_2O_2 (2.8mM), KH_2PO_4/KOH buffer, pH 7.4 (10mM), various concentrations of test drugs and standard (20-320µg/ml) and the pH was then readjusted to 7.4. The ascorbate 100mM was then added to start the reaction. After incubation at 37 °C for 1 h, 1 ml of 1% (w/v) thiobarbituric acid (TBA) in 50mM NaOH and 1ml of 2.8% (w/v) trichloroacetic acid (TCA) was added to the reaction mixture and placed in a hot water bath maintained at 80°C for up to 20 min. The contents were cooled, centrifuged and absorbance of the supernatant was read at 532 nm.

Statistical analysis

Linear regression analysis was used to calculate the IC_{50} values.

Results

Phytochemical Studies

The Preliminary phytochemical studies of methanolic extract of *Bischofia javanica* (BJ) and *Fraxinus floribunda* (FF) showed the presence of alkaloids, flavanoids, terpenoids, tannins and saponins. The chloroform fraction of BJ and FF when undergone for coloumn chromatography, penta cyclic triterpenoids like Friedelin 3-α acetate and β-amyrin were isolated from *Bischofia javanica* and *Fraxinus floribunda* respectively.

Free Radical Scavenging Activities

DPPH Radical Scavenging Assay

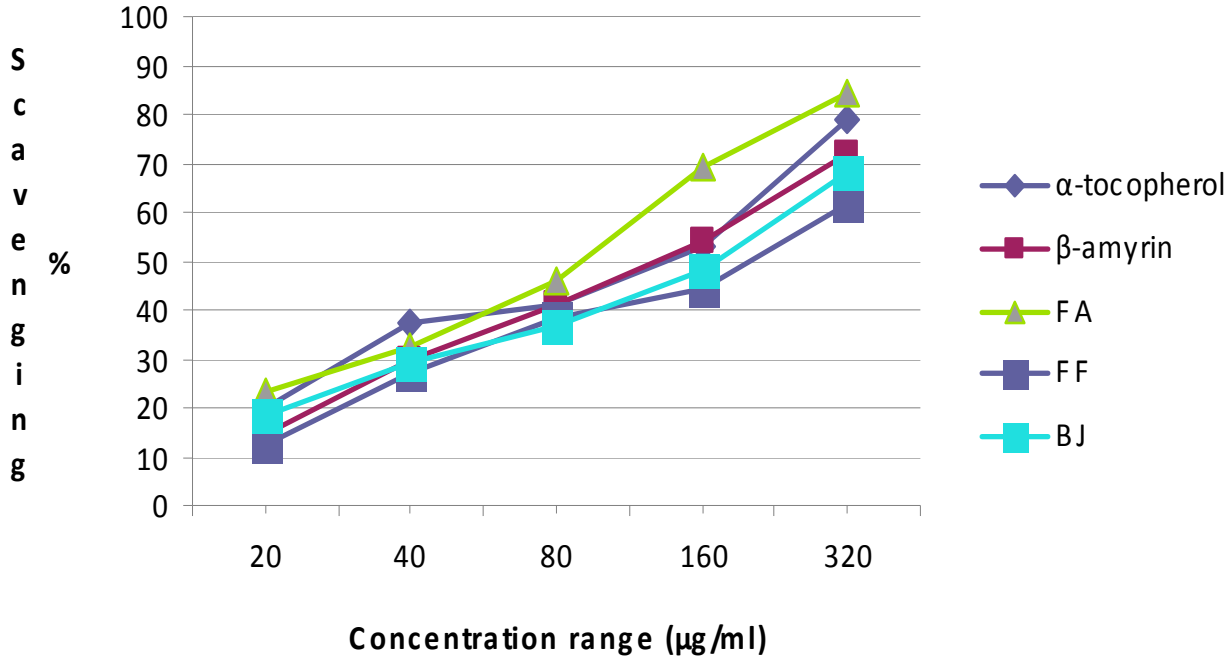
The test drugs showed concentration dependent DPPH radical scavenging activity. Friedelin 3-α-acetate (FA) had shown the highest percentage of scavenging 84.26 compared to positive control α tocopherol 78.67 at 320µg/ml (Figure 1).The IC_{50} values of FA was 118.29µg/ml (Table 1).

Table 1: IC_{50} Values of different test drugs

S.No	Treatment	IC_{50} values (µg/ml)		
		DPPH radical	Lipid Peroxidation	OH radical
1	BJ	188.08	142.00	185.23
2	FF	218.77	172.54	166.63
3	FA	118.29	191.10	163.37
4	β amyrin	168.47	137.90	129.85
5	α tocopherol	150.48	142.22	154.04

BJ=Methanolic extract of *Bischofia javanica*
FF= Methanolic extract of *Fraxinus floribunda,* FA=Friedelin 3 α acetate

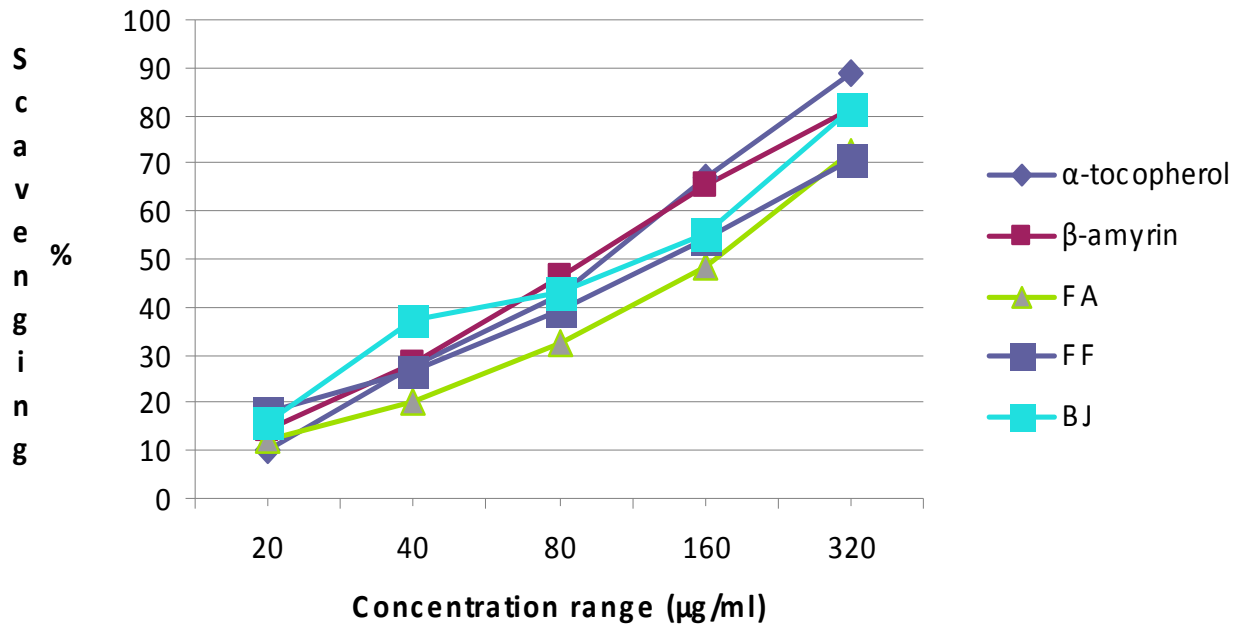
Fig 1: DPPH radical scavenging assay of leaves of *Bischofia javanica* and *Fraxinus floribunda*



Lipid Peroxidation Assay

β amyrin, a pentacyclic triterpenoid isolated from FF showed better scavenging activity compared to other test drugs. The IC_{50} value of β amyrin was 137.90µg/ml (Table 1). The percentage of scavenging was concentration dependent manner for all drugs in the dose range of 20-320µg/ml (Figure 2).

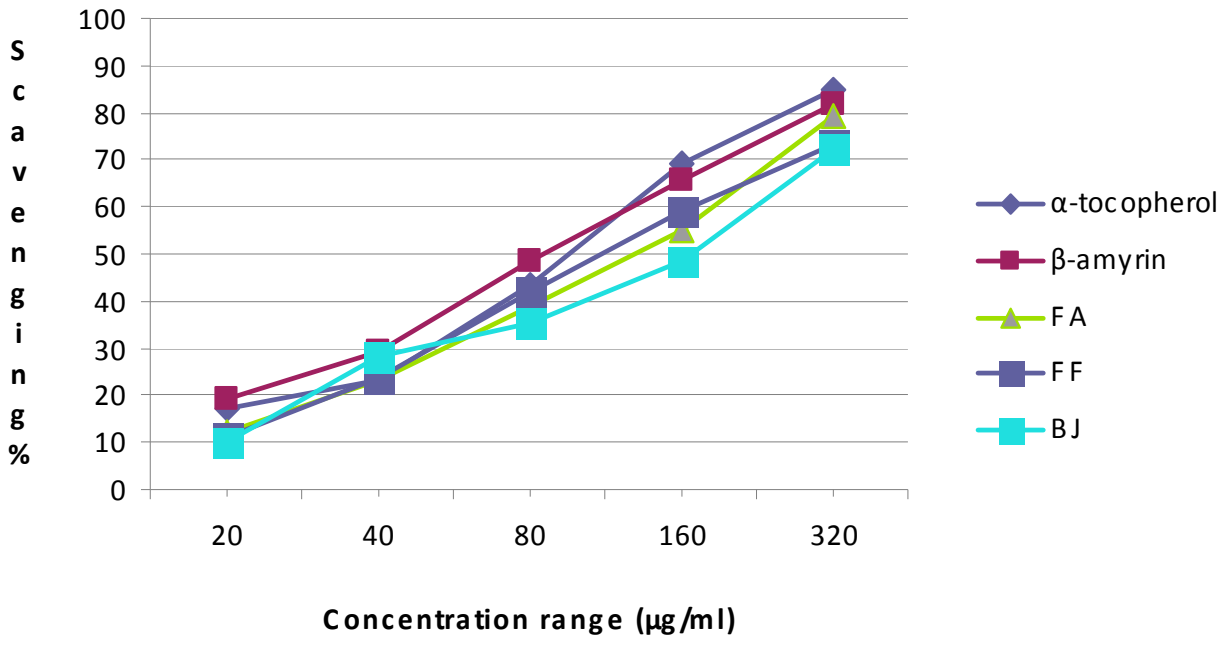
Fig 2: Lipid peroxidation assay of leaves of *Bischofia javanica* and *Fraxinus floribunda*



OH Radical Scavenging Activity

Figure 3 revealed the potent hydroxyl radical scavenging activity of BJ, FF and their isolated products. α tocopherol, the standard drug and β amyrin showed the similar way of scavenging OH radical in the concentration range. Similar to lipid peroxidation assay the β amyrin had the least IC_{50} value (129.85µg/ml) among all the test drugs (Table 1)

Fig 3: Hydroxyl radical scavenging assay of leaves of *Bischofia javanica* and *Fraxinus floribunda*



Discussion

Free radicals include superoxide anion radical, hydrogen peroxide, peroxynitrite, hypochlorous acid/hypochlorite and the extremely reactive hydroxyl radicals. It has been reported that reactive oxygen species such as superoxide anion, hydroxyl radical and peroxynitrite participate in the process of inflammation in various tissues including the skin (31). Synovial fluid from the knee joints of human rheumatoid patients contains increased levels of diene conjugates and TBA-reactive material, suggestive of increased lipid peroxidation in vivo. The possibility that NSAIDs and other anti-inflammatory drugs having multiple mechanisms of action led to search whether they could have anti-oxidant effects. They might directly scavenge such reactive oxidants as hydroxyl radical (OH) and hypochlorous acid (OHCl) (32). Anti-inflammatory and analgesic potency of leaves of *Bischofia javanica* , *Fraxinus floribunda* were studied by the authors in the previous research work (17-18). In this study we have investigated the free radical scavenging activity of leaves of *Bischofia javanica* , *Fraxinus floribunda.* The methanolic extract of leaves of both the plants showed the potent free radical scavenging activities in the *In-vitro* methods like DPPH, Lipid peroxidation and OH radical scavenging activities in the concentration range of 20-320 µg/ml. This led to the bioassay guided fractionation followed by isolation of Phytoconstituents like Friedelin 3-α-acetate (FA) and β amyrin were isolated from *B.javanica* and *F.floribunda* respectively. The IC_{50} values revealed that isolated constituents of BJ and FF were potent scavengers than the methanolic extract. DPPH is a relatively stable radical, it is scavenged by anti-oxidants through the donation of proton forming the reduced DPPH (33). Free radicals induce lipid peroxidation in polyunsaturated lipid rich areas like brain and liver. Initiation of lipid peroxidation by ferrous sulphate takes place through hydroxyl radical by Fenton's

reaction. The inhibition could be caused by absence of ferryl-perferryl complex or by scavenging the hydroxyl radical or the superoxide radicals or by changing the Fe^{3+}/Fe^{2+} or by reducing the rate of conversion of ferrous to ferric or by chelating the iron itself (34). In this study for the first time we explored the free radical scavenging properties of methanolic extracts of *Bischofia javanica* and *Fraxinus floribunda,* Friedelin 3-α-acetate and β amyrin. In conclusion the potent free radical scavenging activities of leaves of *Bischofia javanica* and *Fraxinus floribunda* explored the rationality of the ethnomedicinal utilities of these plants and provided a justification for crosslink mechanism behind anti-inflammatory drugs may have free radical scavenging property.

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